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IDENTIFICATION OF LISTERIA MONOCYTOGENES BY THE CULTURAL AND MORPHOTINCTORIAL NATURE

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Abstract
Listerias are widely in the nature. They were isolated from the soil, water, drainage, plants, vegetal material in decomposition, fodder. L. monocytogenes stood out in the raw food or with insufficient thermal cooking (meat, meat products, fish, shell fish), vegetables, dairy products, and in human or animal healthy carriers. The Liseria type includes gram-positive bacillus, short, with round ends, disposed in palisades or in chains. In old cultures they can appear under the form of long filaments. They are non- sporulated, non-capsulated, discretionary anaerobe mobile at 28ºC. Listeria monocytogenes has a coccobacillary form. Listeria monocytogenes is a pathogen microorganism, with discretionary intracellular habitat. The virulent strains elaborate a hemolysin, listeriolysin O. Other factors of virulence, beside their capacity of intracellular survival, and their wide spread, have a special importance in pathogenesis. Their special capacity to develop or survive in conditions of refrigeration, compared to the main other microorganisms, make the Listeria monocytogenes represent a significant challenge for the area of food production.

Keywords: anaerobe, virulent, filament

INTRODUCTION

The infections with germs of the Listeria type can appear under the form of some sporadic cases or epidemic outbreaks. The last epidemic outbreaks suggest the fact that the listeriosis is in fact a food poisoning, the most frequent way of transmitting the disease being in the present by food.

The most frequently involved food in the epidemic outbreaks is the cabbage, milk, cheese, chicken, turkey, milk, pate, pig tongue, mushrooms etc.

The most severe form of listeriosis is however the maternal-fetal one, not for the mother but for the fetus or the newborn. This can make a precocious form of infection, that is manifested under the form of a generalized septicemia with approx. 40-50% deadly cases, either under the form of late syndrome (7-15 days from birth), characterized by a meningeal syndrome, digestive manifestations and seldom conjunctivitis. Other clinical form signalized in adults and the child at an old age (especially in those with

immune-depression: leukemia, infections with HIV virus) are represented by meningitis, encephalitis, or even septicemias, with a high rate of mortality or with neurological sequelas among the survivors. There were reported also primary cutaneous listeriosis, arthritis, osteomyelitis, intra-abdominal abscesses, peritonitis, pulmonary infections especially in veterinaries and workers from slaughter houses that came in contact with the tissues infected of the sick animals. The diagnosis of laboratory is bacteriological. The serological one can have a value in epidemiologic context.

The pathologic products are harvested depending on the form and localization of the infection: CSF, blood, amniotic liquid, tissular fragments, vaginal secretions, respiratory, tegumentar samples, food samples, salubrity samples etc. and don't need special conditions of transport. In case of prolonged transport the pathologic products will be kept at 35°C in incubator (but not over 48 hours), and for testing that are over 48 hours, the pathologic products will be kept in the refrigerator (at 4°C) or in deepfreezer at -20°C), for the purpose of preventing the contamination with other microorganisms.

MATERIAL AND METHODS

The analytical study was accomplished on pathological products coming from exudates, excrements.

These being the products it was used the technique of seeding for the isolating and identification of Listeria, accomplishing of Gram colored smears from the microbial culture.

1. The technique of seeding for the isolation and identification
   Preparing the necessary material
   - The plates are taken from the refrigerator, are left to reach the room temperature and to get dry in the hood with laminar flow for an hour, with the lid half open
   - The seeding is accomplished in the hood with laminar flow

Method of work

- For each sample it is preferred to have a culture plate
- In a sector of the plate is discharged the loop
- The inoculum is finished with sterile loop tracing parallel lines in the other three scales from the respective half of the plate, seeding thus two bumpers on the plate
- The plates thus seeded are hatched in the thermostat 24 hours, at 37°C.

2. The accomplishing of the Gram colored smear

- It is written on the slide the ID no. of the sample, on the frosted part
- It is placed a drop of physiological serum on the middle of the slide
- It is taken with a sterile ansa a colony of the culture of microorganism to be identified and is placed a drop of serum
- With circular movements of the ansa is created a microbial suspension on the slide, that is spread in a layer as this as possible
- It is left to dry approx. 20-30 minutes
- It is Gram colored as the following:
- The product is fixed passing it through a flame a few time
- It is placed on the coloring bath
- It is colored with a solution of gentian violet or crystal violet for 1 minute
- The colorant is poured and the product is washed with drinking water
- The slide is covered with solution of Lugol for 1 minute
- The colorant is poured and the product is washed with drinking water
- The slide is washed out with a mixture of decolorant alcohol-acetone, by pouring until the poured liquid becomes colorless
- It is washed with drinking water
- It is colored with safranin 2-3 minutes
- The slide is washed with distilled water, it is dried and is examined under the microscope.

RESULTS AND DISCUSSIONS

After 24-48 hours of incubation at 35-37°C the colonies of Listeria have the diameter of 1-1,5mm on the glucose 2% and only 0,2-0,4 mm on the tryptose agar 1-2%. They are round, smooth, easily gibbous, transparent with the aspect of the tear drops. Their center has a glass crystalline aspect, with watery consistency. Examined in the oblique light, at 45º, they appear with blue-green iridescence.

The germs from cultures in the “S” phase are disposed on the smear isolated, grouped in palisades, and for those of “R” phase, appear more frequently coccobacili disposed in short chains of 3-5 elements.

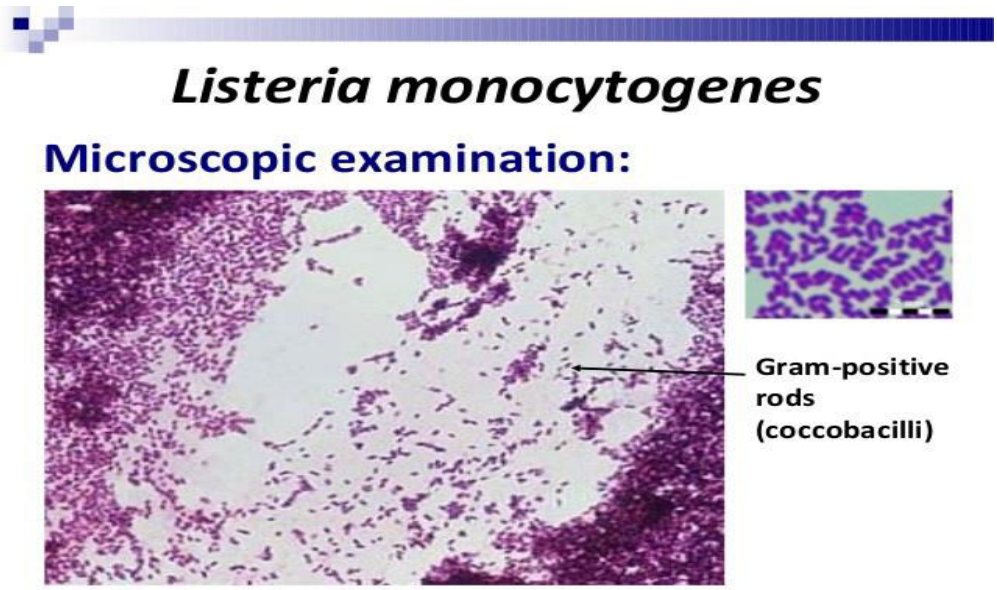

Fig. 1. *Listeria monocytogenes*Grame positive

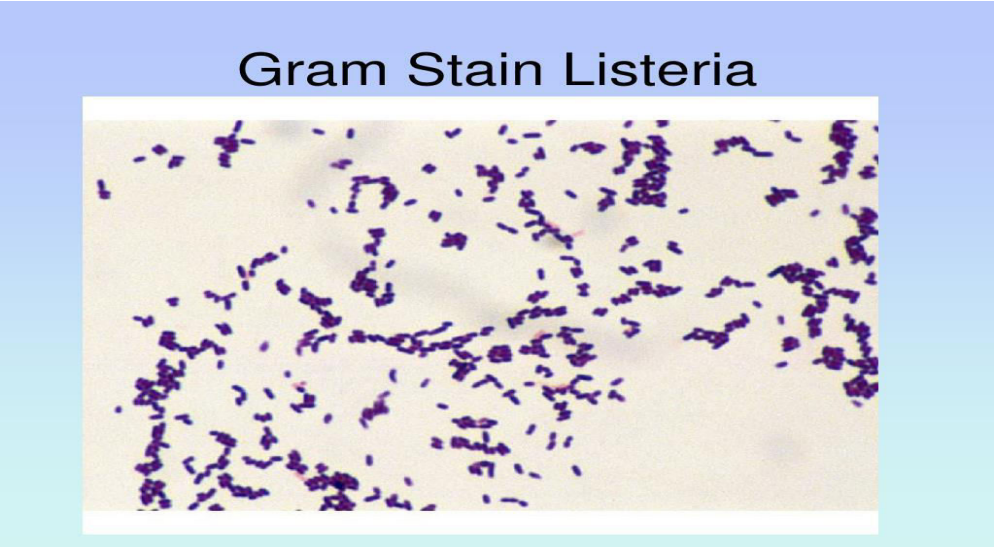

Fig. 2. *Listeria monocytogenes* Grame positive

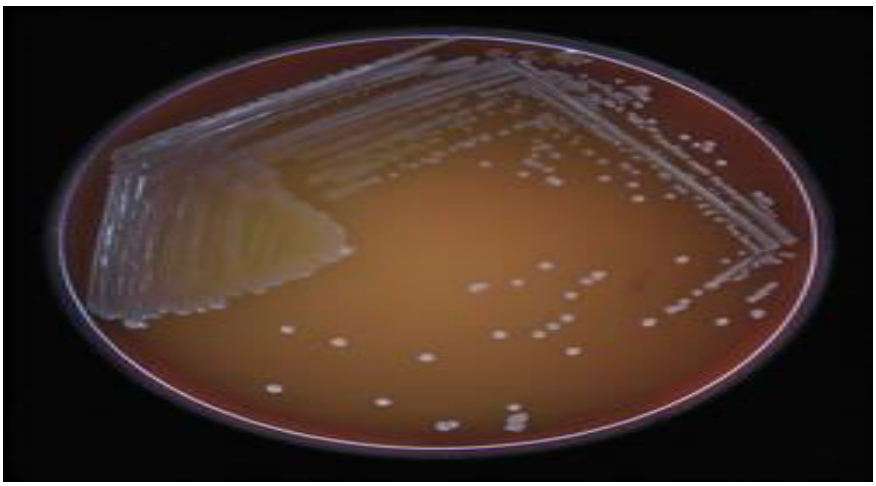

Fig.3**.** *Listeria monocytogenes.* Blood Agar
Culture 24 hours

The isolation from the contaminated pathological samples is difficult, it seeds a part o sample in 9 parts of broth of enriching with nalidixic acid.

The listeria are short bacilli, right or curved, most of the times with coccobacilar or cocal form, with round ends and dimensions of 0,5-2µm length and 0,8µm depth, in the pathologic samples they appear either extracellular or phagocyte, the indubitable test o listeriosis.

In the young cultures, incubated at 35-37°C, are dominating the short forms, coccobacilar, while in the old cultures the polymorphism appears, most often with long filament forms of 6-20µm similar to lactobacilli. They are colored gram-positive on the smears accomplished from young cultures and can be gram-negative in the old cultures. In a prolonged discoloration, of over 5 minutes, over 50% of the germs, even those coming from young cultures, can become gram-negative.

The colonies of the rugose forms have the matt center, are large, oblate, with irregular margins, with central crateriform depression, friable, hard to be emulsified. After the pricking-out the colonies of Lisyteria have left "imprints" on the agar.

On agar with 5% ram blood, the colonies of Listeria monocytogenes are surrounded with a narrow area of β diffuse hemolysis and those of Listeria seeligeri are weakly hemolytic. And those of Listeria *ivanovii* form a wider area of β hemolysis, that after 36-48 hours have the aspect of double or even triple hemolysis.

The cultures of Listeria monocytogenes on solid medium have spread a characteristic smell of acidulate milk. In the semi-solid mediums as the agar 3‰, after the seeding by stinging and incubation at the room temperature, Listeria monocytogenes grows under the form of an umbrella at 3-5 mm from the surface of the agar proving an important mobility.

Other methods that were used for the detecting of the species of Listeria, were showed up in the study "On the Specificity of PCR Detection of *Listeria monocytogenes* in Food: a Comparison of Published Primers", made by R.Aznar[ab],B.Alarcón[ab]. First of all the authors have accomplished a polyphase approach to establish a collection of reference strains. These were characterized biochemically and genetically by API-Lis and PCR, polymorpheus randomly amplified (RAPD-PCR), respectively. The random amplifying of DNA was accomplished with the universal primers M13, T7 and T3 and was created a data bank in order to compile the RAPD patterns of all the analyzed strains. The analysis of the UPGMA cluster of the RAPD profiles with primer M13 showed eight clusters with 72,3% similarity. The clusters 2 and 7 corresponded to L. monocytogenes. The clusters 1 and 6

groups of strains of L. ivanovii. The clusters 3, 4, 5 and 8 coresponded to L. grayi, L. innocua, L. welshimeri and, L. seeligeri, respectively. The analysis of the model has underlined the existence of the reference strains wrongly identified, which was confirmed by the analysis of the sequence of ADNc 16S. RAPD-PCR is a fast genetic test that helped the confirmation of the identity of the strains. Based on the results of the PCR specificity, the primers LM1 – LM2 were the best combination for the detection of L. monocytogenes because they amplified only the specific fragment in the strains that were evaluated genetically and biochemically as belonging to the species. The specificity of other primers analyzed is discussed.

CONCLUSIONS

For the differentiation of the Listeria type from other similar types it is tested the anaerobe discretionary growth, the mobility in the wet product or in the column of soft agar of the culture at the room temperature, the growth at temperatures between 2℃ and 42℃, the production of oxidase, catalysis, urease, H2S in the TSI medium, the fermenting of the glucose.

The identification up to the level of species is very important because all the listeria can contaminate the food, but only *L. monocytogenes* has clinical significance and exceptional L. *ivanovii* or *L. seeligeri*.

The hemolysis is essential for the differentiation of L. monocytogenes from L. inocua, because of the tight affinity of the nine species and overlapping of many phenotype kinds.

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